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DEVELOPMENT OF OPTICAL BIOSENSOR TO DETERMINE BENZOIC ACID CONCENTRATION BASED ON ITS INHIBITIVE EFFECT ON TYROSINASE ENZYME

HAIRUL HISHAM HAMZAH

FS 2010 29

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HAIRUL HISHAM HAMZAH

MASTER OF SCIENCE

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2010

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By

HAIRUL HISHAM HAMZAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, In Fulfilment of the Requirements for the Degree of Master of Science

September 2010

UPM

DEDICATIONS

To Assoc. Prof. Dr. Nor Azah Yusof,
for her patience, guidance, understanding and trust….

To Prof. Dato' Dr. Abu Bakar Salleh and Assoc. Prof. Dr. Fatimah Abu Bakar,
for their patience and trust….

To my beloved mom and family,
for their love, support and concern….

To all my friends,
for their support and understanding….

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science.

DEVELOPMENT OF OPTICAL BIOSENSOR TO DETERMINE BENZOIC ACID CONCENTRATION BASED ON ITS INHIBITIVE EFFECT ON TYROSINASE ENZYME

By

HAIRUL HISHAM BIN HAMZAH

September 2010

Chairman : Associate Professor Nor Azah Yusof, PhD

Faculty : Science

An optical biosensor using phenol biosensor for the determination of benzoic acid based on inhibitive effect of benzoic acid on tyrosinase enzyme has been developed in this study. Two methods were developed which were based on in-solution tyrosinase and immobilized tyrosinase. Tyrosinase enzyme, phenol and 3-methyl-2-benzothiazolinone hydrazone (MBTH) were used as reagents for determination of benzoic acid. In-solution and immobilized tyrosinase as sensing element were successfully applied for the determination of benzoic acid.

In the detection of benzoic acid based on in-solution tyrosinase, the solution changed its color from dark maroon to light maroon depending on the concentration of benzoic acid, due to the inhibition of tyrosinase reaction by benzoic acid with response time of thirty minutes. The absorbance of products as the output of enzymatic reaction was detected by using uv-visible spectrophotometer with maximum absorbance at 504 nm. The biosensor demonstrated optimum activity at pH 7. The relative standard deviation (RSD) of the reproducibility of this method was

very good with RSD value of 1.91 %. The dynamic range of benzoic acid concentration was found to be between 50 ppm to 700 ppm with the detection limit (LOD) of 0.19 ppm. The kinetic parameters Michaelis-Menten constant (K_M) and maximum absorbance (A_{max}) in the absence and presence of benzoic acid showed the inhibition of benzoic acid on tyrosinase activity is competitive inhibitor with the inhibition constant (K_i) 90.9 ppm.

Test strip for detection of benzoic acid was developed by immobilizing tyrosinase, phenol and MBTH into filter paper using polystyrene as a polymeric support. The sensing scheme was based on the decreasing intensity of maroon color of test strip when dipped into benzoic acid solution. The test strip was characterized using optical fiber reflectance spectrometer (OFRS) and the result showed the test strip had a maximum reflectance at 375.65 nm. The optimum response of biosensor was achieved at pH 7. A linear response of the biosensor was obtained in the benzoic acid concentration range of 50 ppm to 700 ppm with LOD of 0.28 ppm. The reproducibility of the biosensor was good with calculated RSD of 0.47 %. The kinetic analyses showed that the inhibition of benzoic acid on tyrosinase activity was reversible and competitive inhibition with K_i of 52.9 ppm. The activity of the developed test strip was fairly sustained during for 20 days when stored at 3° C.

The results obtained from both methods developed in this study were compared with the established method of high performance liquid chromatography (HPLC). Excellent agreement was obtained between the developed and the HPLC methods.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains.

PEMBANGUNAN PENDERIABIO OPTIK UNTUK PENENTUAN KEPEKATAN ASID BENZOIK BERDASARKAN KESAN PERENCATANNYA KE ATAS ENZIM TIROSINASE

Oleh

HAIRUL HISHAM HAMZAH

September 2010

Pengerusi : Profesor Madya Nor Azah Yusof, PhD

Fakulti : Sains

Penderiabio optik mengunakan penderiabio fenol untuk penentuan asid benzoik berdasarkan kesan perencatan asid benzoik ke atas enzim tirosinase telah dibangunkan. Dua kaedah dibangunkan adalah yang mana berdasarkan tirosinase dalam larutan dan terpegun tirosinase. Enzim tirosinase, fenol, dan 3-metil-2-benzotiozolinon hidrazon (MBTH) digunakan sebagai bahan tindak balas untuk penentuan asid benzoik. Tirosinase dalam larutan dan terpegun sebagai elemen pengesanan telah berjaya diaplikasikan untuk penentuan asid benzoik.

Dalam penentuan asid benzoik berdasarkan tirosinase dalam larutan, larutan bertukar warna dari warna merah bata gelap kepada warna merah bata cerah bergantung kepada kepekatan asid benzoik merujuk kepada perencatan tindak balas tirosinase oleh asid benzoik dengan masa tindak balas selama 30 minit. Penyerapan produk sebagai hasil dari tindak balas tirosinase ditentukan menggunakan spektrofotometer ultra-nampak dengan penyerapan maksimum 504 nm. Penderiabio menunjukkan aktiviti optimum biosensor ialah pada pH 7. Sisihan piawai relatif

(RSD) bagi kebolehulangan kaedah ini adalah sangat baik dengan nilai RSD sebanyak 1.91%. Julat kepekatan dinamik bagi kepekatan asid benzoik ialah 50 ppm hingga 700 ppm dengan had pengesanan (LOD) adalah pada 0.19 ppm. Parameter kinetik untuk pemalar Michaelis-Menten (K_M) dan penyerapan maksimum (A_{max}) dalam ketidakhadiran dan kehadiran asid benzoik menunjukkan bahawa perencatan asid benzoik ke atas aktiviti tirosinase adalah perencat bersaing dan berbalik dengan pemalar perencatan (K_i) ialah 90.9 ppm.

Kepingan ujian untuk pengesanan asid benzoik dibangunkan dengan memegunkan tirosinase, fenol, dan MBTH ke dalam kertas turas menggunakan polistirina sebagai polimer sokongan. Skima pengesanan adalah berdasarkan pengurangan keamatan warna merah bata ke atas kepingan ujian apabila dicelup ke dalam larutan asid benzoik. Kepingan ujian ini dicirikan menggunakan spektrofotometer gentian optik (OFRS) dan keputusan menunjukkan bahawa kepingan ujian menpunyai pantulan maksimum pada 375.65 nm. Penderiabio mempunyai aktiviti optimum yang dicapai pada pH 7. Gerak balas linear penderiabio diperolehi dalam julat kepekatan asid benzoik adalah 50 hingga 700 ppm dengan had pengesanan adalah 0.28 ppm. Kebolehulangan penderiabio adalah baik dengan RSD ialah 0.47 %. Analisis kinetik menunjukkan bahawa perencatan asid benzoik ke atas enzim tirosinase adalah perencat bersaing dengan K_i ialah 52.9 ppm. Aktiviti kepingan ujian kekal selama 20 hari apabila disimpan pada suhu 3° C.

Keputusan yang diperolehi daripada kedua-dua kaedah yang dibina dalam kajian ini telah dibandingkan dengan kaedah yang diiktiraf iaitu kromatografi cecair prestasi tinggi (HPLC). Persetujuan yang sangat baik telah dicapai antara kaedah yang dibina dan kaedah HPLC.

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Finally, my special thanks are dedicated to my family for their never ending encouragement, support, advice, understanding and love.

I certify that a Thesis Examination Committee has met on 28 September 2010 to conduct the final examination of Hairul Hisham bin Hamzah on his Master of Science thesis entitled **"Development of Optical Biosensor to Determine Benzoic Acid Concentration Based on Its Inhibitive Effect on Tyrosinase Enzyme"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the (Master of Science).

Members of the Thesis Examination Committee were as follows:

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Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

_____

HAIRUL HISHAM HAMZAH

Date: 28 September 2010

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